

October 20, 2005
US Serial No. 10/035,215
Reply to June 21, 2005 Office Action

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims

1. A method for the propagation ~~micropropagation in tissue culture of~~ plantlets of *Arundo donax*, comprising:
 - (a) obtaining explant material from meristematic tissue, juvenile or immature *Arundo donax* plant structures;
 - (b) cleaning said explant material to obtain aseptic plant material;
 - (c) introducing said aseptic explant material into a semi-solid or solid embryo induction medium, allowing for the production of mature embryos;
 - (d) culturing the mature embryos on semi-solid or solid germination medium to thereby generate said plantlets; and
 - (e) transferring plantlets generated from step (d) or nodal segments thereof to a solid or semi-solid shoot multiplication medium to obtain multiple shoots from the plantlets to trays which will float on a liquid medium in a float bed nursery apparatus in a non-aseptic manner and which float bed nursery provides conditions conducive to growth and multiplication of the plantlets, and placing said trays at one end of said apparatus; and
 - (f) moving the trays of plantlets along a length of the float bed apparatus at a periodic interval, such that when the trays reach the opposite end of the apparatus the plantlets have multiplied and grown sufficiently to reach a maturity of plants that are ready to be planted in the field,

whereby a plurality of *Arundo donax* plants is obtained.

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2. (original) The method of claim 1, further comprising transferring the mature embryos from step (c) to a liquid suspension culture medium to thereby induce the production of more embryos prior to step (d).

3. (currently amended) The method of claim 4 2, further comprising ~~transferring the mature embryos from step (c) to a liquid suspension culture medium to thereby induce the production of more embryos, followed by~~ splitting and subculturing the resultant multiple embryos in fresh liquid suspension medium to induce further embryo multiplication, prior to step (d).

4. (cancelled) .

5. (cancelled).

6. (currently amended) The method of claim 1, wherein the embryo induction medium comprises ~~a basal plant~~ LS or MS medium supplemented with sucrose, a gelling agent, and one or more of 2,4-dichlorophenoxyacetic acid (2,4-D), 6-benzyladenine (BA), indoleacetic acid (IAA), kinetin (K), and thidiazuron (TDZ).

7. (cancelled).

8. (currently amended) The method of claim 7 6, wherein the embryo induction medium comprises LS medium supplemented with IAA, 2,4-D and sucrose.

9. (original) The method of claim 8, which contains 1.0 mg/L IAA, 2 mg/L 2,4-D and 20 g/L sucrose.

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10. (currently amended) The method of claim 1, wherein the germination medium comprises a ~~basal plant~~ LS or MS medium supplemented with sucrose and a gelling agent.

11. (cancelled).

12. (currently amended) The method of claim 2, wherein the liquid suspension culture medium comprises a ~~basal plant~~ LS or MS medium supplemented with sucrose, and one or more of 2,4-dichlorophenoxyacetic acid (2,4-D), 6-benzyladenine (BA), indoleacetic acid (IAA), kinetin (K), and thidiazuron (TDZ).

13. (currently amended) The method of claim 12, wherein the liquid suspension culture medium comprises one or more of 1 – 6 mg/L 2,4-D, 0.5 – 2 mg/L BA, ~~1 – 3 mg/L BA~~, 1 – 3 mg/L K, and 0.05 – 1.0 mg/L TDZ.

14. (original) The method of claim 12, wherein the liquid suspension culture medium further comprises asparagine.

15. (currently amended) The method of claim 4 ~~57~~, wherein the shoot multiplication medium comprises a ~~basal plant~~ LS or MS medium supplemented with sucrose, a gelling agent, and one or more of 2,4-dichlorophenoxyacetic acid (2,4-D), 6-benzyladenine (BA), indoleacetic acid (IAA), kinetin (K), and thidiazuron (TDZ).

16. (original) The method of claim 15, wherein the shoot multiplication medium comprises MS medium supplemented with Gamborg's vitamins, BA, TDZ and sucrose.

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17. (original) The method of claim 16, which contains 1.0 mg/L BA, 0.05 mg/L TDZ and 30 g/L sucrose.

Claims 18 – 55 (cancelled).

56. (new) The method of claim 1, wherein the plants are allowed to grow and multiply in the float bed apparatus for about 30 days to reach such maturity of plants that are ready to be planted in the field

57. (new) The method of claim 1, wherein additional plantlets for transferring to trays are generated between steps (d) and (e) by transferring plantlets or nodal segments thereof from step (d) to a solid or semi-solid shoot multiplication medium to obtain multiple shoots from the plantlets.

58. (new) The method of claim 1, further comprising obtaining plant material from maturing plants in the float bed and returning the plant material to a float tray to start the multiplication and growth of plants anew in the float bed apparatus, thereby reducing or eliminating the need for plantlets obtained from steps (a) through (d).

59. (new) The method of claim 58, wherein said plant material is obtained from maturing plants in the float bed apparatus at a stage where the plants contain at least one node, and whereby the plant material is obtained by clipping off the tops of the maturing plants containing the at least one node.

60. (new) The method of claim 58, wherein the plant material is obtained at about the 22nd to the 26th day in the float bed apparatus.

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61. (new) The method of claim 59, wherein the tops containing at least one node are treating with plant hormones when introduced anew to the float bed apparatus.

62. (new) The method of claim 1, wherein new trays of plantlets are added to the float bed apparatus when trays with mature plants are removed at the opposite end, thereby operating in a continuous, conveyor belt fashion.

63. (new) The method of claim 1, wherein the periodic interval is daily.

64. (new) The method of claim 1, wherein the float bed apparatus for the propagation of plants comprises a bottom frame structure of about six inches high, which is constructed on a level smooth base to form a device to hold a liquid medium at a depth of about four to six inches, and onto which a canopy framework of a sufficient height to accommodate the plants is attached to the bottom frame.

65. (new) The method of claim 64, wherein the bottom frame structure is lined with a plastic film of about 6 mils thickness to retain the liquid medium in the float bed.

66. (new) The method of claim 64, wherein the canopy framework is constructed of plastic pipe.

67. (new) The method of claim 64, wherein at least a portion of one end of the canopy framework under which plantlets are transferred to the float bed apparatus is covered with a material of sufficient light reduction characteristics to allow growth but to protect plantlets from wilting and to acclimatize newly transplanted plantlets.

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68. (new) The method of claim 64, which further comprises an overhead misting apparatus with emitters spaced along the linear dimension of the canopy framework to attain desired humidity.

69. (new) The method of claim 64, wherein the bottom frame structure contains water that is supplemented with nutrients and plant hormones conducive to plant growth and multiplication.

70. (new) A method for the propagation of *Arundo donax* to obtain plants thereof, comprising:

- (a) obtaining plantlets of the *Arundo donax* by somatic embryogenesis or from portions of mature plants thereof containing at least one node;
- (b) transferring said plantlets or nodal material to trays which will float on a liquid medium in an Nth float bed module that provides conditions conducive for growth and multiplication of the plantlets or nodal material;
- (c) placing the Nth float bed module at the end of a pathway containing N -1 float bed modules, said pathway permitting movement of the Nth float bed from a first position to a second position; and
- (d) permitting the Nth float bed module to move along the pathway so as to permit the Nth float bed module to arrive at the second position when the plantlets are sufficiently mature for planting proximate to the second position.

71. (new) The method of claim 70, wherein the Nth float bed is transported from the second position to the first position for introduction of a new tray of plantlets when the tray with mature plants is removed at the second position, thereby permitting the method to operate in a continuous, conveyor belt fashion.